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Prediction of the Binding Mode of Imidacloprid and Related Compounds to House-Fly Head Acetylcholine Receptors Using Three-Dimensional QSAR Analysis

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Abstract: The binding activity of imidacloprid and related compounds to nicotinic acetylcholine receptors (nAChR) of house flies was measured by use of radioactive α-bungarotoxin as a ligand. Variations in the activity were examined three-dimensionally using comparative molecular field analysis (CoMFA). The CoMFA results suggest that one conformer among the four stable ones is active and provide support for one of the proposed binding models for this class of compound, in which the nitrogen atom of the pyridine ring and the nitrogen atom at the 1-position of the imidazolidine ring interact with the hydrogen-donating and electron-rich sites of nAChR, respectively. The CoMFA field map showed that the nitroimino moiety and a portion of the imidazolidine ring were mainly surrounded by a sterically and electrostatically sensitive region of nAChR. © 1998 Society of Chemical Industry

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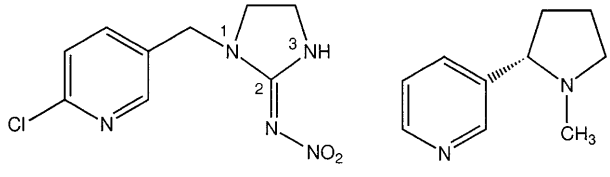
Key words: insecticide; nitromethylene compounds; neonicotinoid; nicotinic acetylcholine receptor; binding activity; quantitative structure–activity relationship; CoMFA

1 INTRODUCTION

Nitromethylene insecticides, including imidacloprid (Fig. 1), have recently been developed as a new generation of pest control agents which act on the insect nervous system.[1–4] This class of compounds is often referred to as neonicotinoids and is characterized by persistent effects, broad spectra, good systemic properties, low degree of toxicity to mammals and aquatic life, and stability in the fields.

Neonicotinoids act at the acetylcholine binding site of the nicotinic acetylcholine receptor (nAChR) of insects.[5–13] The insecticidal activity of imidacloprid is about 10 000-fold higher than the natural insecticide, *l*-nicotine (Fig. 1).[8,9] Using a series of analogous compounds, some positive relationships have been found which correlate the insecticidal and knockdown activities with binding activity to nAChR.[8] We have also shown that the insecticidal activity of imidacloprid and



Imidacloprid *l*-Nicotine

Fig. 1. Structures of imidacloprid and *l*-nicotine.

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related compounds against American cockroaches (*Periplaneta americana* L.) increases with neuroexcitatory activity and hydrophobicity.[14]

From these structure–activity studies, two binding models have thus far been proposed for such compounds. Yamamoto *et al.*[9] proposed that the nitrogen atom of the pyridine ring and the nitrogen atom at the 1-position of the imidazolidine ring interact with the hydrogen-donating and electron-rich sites of nAChR, respectively, because the distance between these two nitrogen atoms is very similar to the distance between the two nitrogen atoms on *l*-nicotine (Fig. 2(a) and (b)). Kagabu[15] proposed that the nitrogen atom at the 1-position of the imidazolidine ring and one of the oxygen atoms of the nitro group play an important role in the interaction with the binding sites on nAChR (Fig. 2(c)).

In this study, we report some measurements of the binding activity of imidacloprid and related compounds to nAChR prepared from house-fly (*Musca domestica* L.) heads, relative to the inhibition of the binding of (3-[^{125}I]*iodotyrosyl*^{54})α-bungarotoxin ([^{125}I]α-BGTX). In order to understand better the structural requirements, binding activity was analysed using comparative molecular field analysis (CoMFA),[16–18] which is a technique for the analysis of three-dimensional quantitative structure–activity. In the analysis, four series of conformers of the compounds, i.e. two series from two structures, based on the X-ray crystallographic data of imidacloprid[19] and two other series from respective 180° rotamers with respect to the pyridine ring, were considered. The best CoMFA result supported the binding model of Yamamoto *et al.*[9] We also show that a specific chemical structure of the compounds and a specific conformation of the nitroimino moiety are required for a successful steric and electrostatic interaction with nAChR.

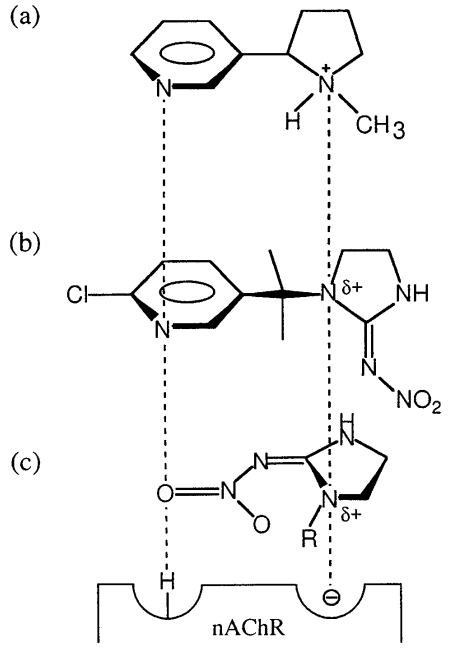


Fig. 2. Proposed interactions of (a) *l*-nicotine and (b, c) imidacloprid with nAChR. R in (c) is 6-Cl-pyridylmethyl. Model (b) was proposed by Yamamoto *et al.*[9] and model (c) by Kagabu.[15]

2 EXPERIMENTAL

2.1 Chemicals

The test chemicals listed in Table 1 were kindly provided from Nihon Bayer Agrochem Co. [^{125}I]α-BGTX (74 TBq mmol^{-1}) was purchased from Amersham International, Buckinghamshire, UK. BCA protein assay reagents were purchased from Pierce, Rockford, IL, USA. Other chemicals were from Wako Pure Chemical Industries, Osaka, Japan, Nacalai Tesque, Kyoto, Japan and Aldrich Chemical Co., Milwaukee, WI, USA.

2.2 Binding assay

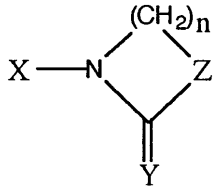
2.2.1 nAChR preparation

A house-fly head membrane fraction was prepared using the general method described by Liu *et al.*[10–12] House-fly heads were collected by sieving house flies which had been frozen by liquid nitrogen. The head preparation (1 mg) was homogenized three times in sodium phosphate (100 mM, pH 7·4; 5 ml) containing sucrose (0·32 M) and EDTA (0·1 mM), with a Polytron for 30 s each with a 60-s interval. The homogenate was filtered through three layers of cheesecloth to remove debris. The filtrate was centrifuged at 700*g* for 10 min, and the supernatant was then further centrifuged at 125 000*g* for 60 min. The pellet was suspended in the binding assay buffer (pH 7·4) of sodium chloride (50 mM) and sodium phosphate (10 mM) containing Triton X-100 (1 g litre^{-1}). This membrane preparation was immediately used for the binding assay or stored at −80°C for periods of up to two weeks. The protein concentration was determined by using BCA protein assay reagent.[20]

2.2.2 Binding assay

The nAChR preparation (*c.* 250 μg protein) was incubated at 25°C for 60 min with [^{125}I]α-BGTX (0·2 nM) and test chemicals at appropriate concentrations (*c.* $1 \times 10^{-2} \sim 10^{-9}$ M) in the binding assay buffer (200 μl). The reaction was terminated by rapid filtration through an Unifilter GF/B (Packard Instrument Co., Meriden, CT, USA) which had been treated with 0·1% polyethylenimine to prevent the nonspecific binding of [^{125}I]α-BGTX to the filter.[21] The filters were rinsed three times with a buffer (pH 7·4) composed of sodium chloride (50 mM) and sodium phosphate (10 mM), once with methanol, and then dried. The radioactivity was determined using a Topcount (Packard) instrument,

TABLE 1
Binding Activity of Imidacloprid and Related Compounds



Compound					*log* (*1*/K_i)		
No.	*X*	*Y*	*Z*	*n*	*Obsd*	*Calcd*[a]	Δ
1	3-Pyridylmethyl	$CHNO_2$	NH	2	7·30	6·89	0·41
2	6-Cl-3-pyridylmethyl	$CHNO_2$	NH	2	7·49	7·03	0·46
3	6-Me-3-pyridylmethyl	$CHNO_2$	NH	2	6·72	6·90	−0·18
4	6-Cl-3-pyridyl	$CHNO_2$	NH	2	4·47	4·18	0·29
5	2-(6-Cl-3-pyridyl)ethyl	$CHNO_2$	NH	2	4·71	4·97	−0·26
6	2-Pyridylmethyl	$CHNO_2$	NH	2	4·84	5·34	−0·50
7	4-Pyridylmethyl	$CHNO_2$	NH	2	3·14	2·96	0·18
8	Benzyl	$CHNO_2$	NH	2	5·49	6·46	−0·97
9	4-Cl-benzyl	$CHNO_2$	NH	2	5·30	5·48	−0·18
10	5-(2-Cl-thiazolyl)methyl	$CHNO_2$	NH	2	6·97	6·84	0·13
11	3-Pyridylmethyl	NNO_2	NH	2	5·35	5·50	−0·15
12[b]	6-Cl-3-pyridylmethyl	NNO_2	NH	2	6·00	5·56	0·44
13	6-Cl-3-pyridylmethyl	NNO_2	NMe	2	3·83	4·12	−0·39
14	6-Cl-3-pyridylmethyl	NCN	NH	2	5·09	4·48	0·61
15	6-Cl-3-pyridylmethyl	CHCN	NH	2	5·11	5·42	−0·31
16	6-Cl-3-pyridylmethyl	$CHNO_2$	CH_2	2	7·11	6·99	0·12
17	6-Cl-3-pyridylmethyl	$CHNO_2$	O	2	6·40	6·34	0·06
18	6-Cl-3-pyridylmethyl	$CHNO_2$	S	2	7·00	7·01	−0·01
19	6-Cl-3-pyridylmethyl	$CHNO_2$	NH	3	7·70	7·60	0·10
20	*l*-nicotine				6·03[c]	6·01[d]	0·02
21	*l*-anabasine				5·71[c]	5·77	−0·06
22	cytisine				6·73[c]	6·63	0·10

[a] By eqn (11) in Table 2.
[b] Imidacloprid.
[c] Estimated by eqn (1).
[d] For *R*-configuration (Fig. 4).

after adding 30 μl of Microscinti-O (Packard). Specific binding was defined as the difference in radioactivities measured in the absence (10 000 ~ 15 000 cpm) and presence (2000 ~ 3000 cpm) of 10 μM unlabelled α-BGTX. The saturation curve was obtained by using only [^{125}I]α-BGTX at various concentrations.

2.2.3 *Data calculation*

IC_{50} values, the molar concentrations required for 50% inhibition of the specific binding of [^{125}I]α-BGTX, were determined by a nonlinear regression analysis using PRISM.[22] The binding activity of test chemicals **1**–**19** was evaluated by calculating K_i using the following equation:[23]

$$K_i = IC_{50}/(1 + [L]/K_d),$$

where $[L]$ is the concentration of the radiolabeled ligand and K_d is the dissociation constant, which was determined to be 0·058 nM by nonlinear regression analysis from the saturation curve of [^{125}I]α-BGTX to nAChR using PRISM. The $\log(1/K_i)$ values, which are listed in Table 1, were taken as the index of the binding activity.

For three natural nAChR agonists, *l*-nicotine, *l*-anabasine and cytisine, which bind *via* a mechanism similar to imidacloprid, the $\log(1/K_i)$ values were estimated by eqn (1) obtained from compounds **1**–**19**, where $\log(1/K_i)_T$ values were determined by Tomizawa *et al.*[7,8]

$$\log(1/K_i) = 1{\cdot}037(\pm 0{\cdot}144)\log(1/K_i)_T - 0{\cdot}192(\pm 0{\cdot}846) \quad (1)$$

$$n = 19,\ s = 0{\cdot}352,\ r = 0{\cdot}965,\ F(1, 17) = 232.1$$

In this and the following equations, n represents the number of compounds, s the standard deviation, r the correlation coefficient, and F the ratio of regression and residual variances. Figures in parentheses are the 95% confidence intervals of the regression coefficient and the intercept. The calculated values for these three compounds are listed in Table 1.

2.3 CoMFA

2.3.1 Molecular modelling

All computations were done with the molecular modelling software package SYBYL, version 6.2.[24] To select initial conformations of the compounds, the X-ray crystallographic data of imidacloprid (**12**) were utilized.[19] Two types of structure having different dihedral angles between the two rings were found in crystals. Structures of this and other compounds were constructed as described below and were fully optimized by the semiempirical molecular orbital method, either AM1[25] or PM3,[26–28] to give relatively stable conformations. For the optimized coordinates, atomic charges were calculated using AM1. The structure optimization of some key compounds, such as the two types of structure of imidacloprid, was carried out using PM3, giving imidacloprid-**I** and -**II** (Fig. 3). Since the 180° rotamers of imidacloprid-**I** and -**II** with respect to the pyridine ring were also assumed to be stable, these were optimized by PM3 to give imidacloprid-**III** and -**IV** (Fig. 3).

The other compounds used in the study were separately modelled from these four conformers, with the result that four series of conformers (**I**–**IV**) for each compound were used in the study. For compounds **1**–**10** and **16**–**19**, the nitroimino group of imidacloprid (**12**) was replaced by a nitromethylene group, and for com-

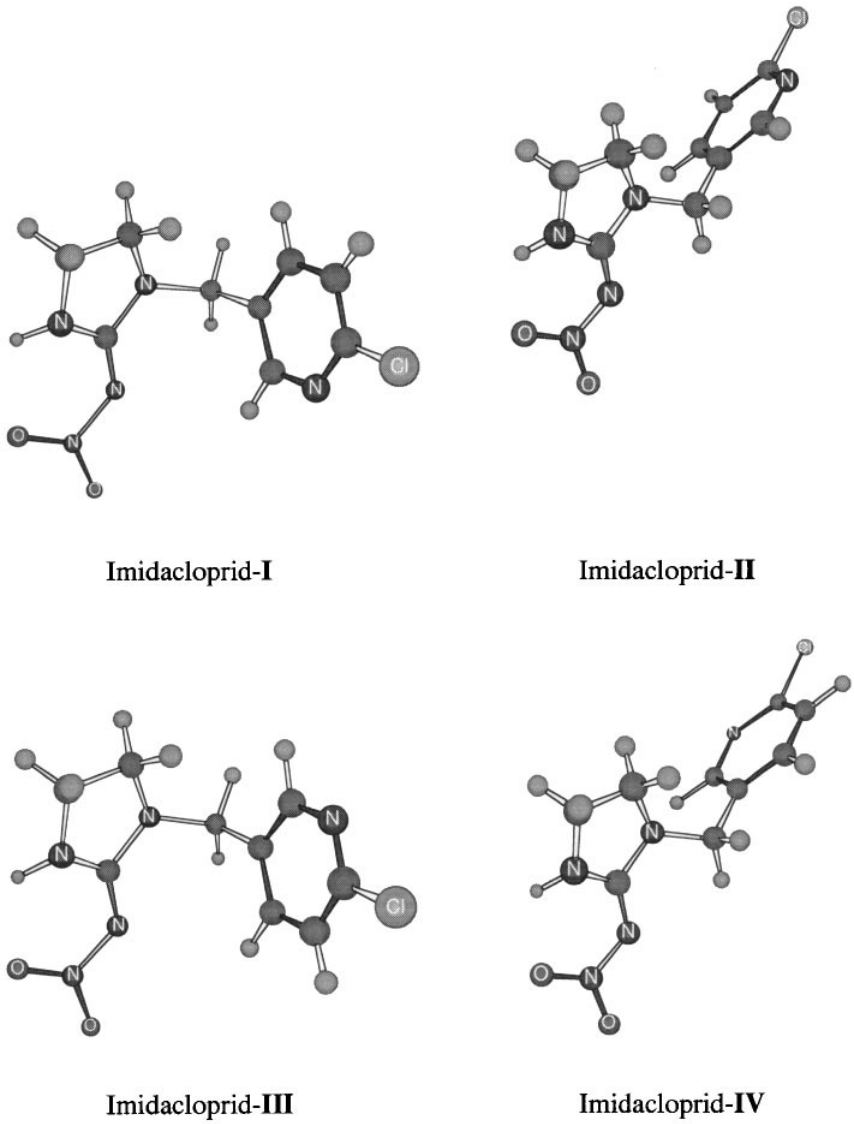


Fig. 3. Four optimized conformations of imidacloprid. Two different crystal structures (**I**, **II**) and their rotamers with respect to the pyridine ring (**III**, **IV**, respectively) were fully optimized by the semiempirical molecular orbital method, PM3.

pounds **14** and **15**, by a cyanoimino and a cyanomethylene group, respectively. The 6-Cl-pyridylmethyl moiety of imidacloprid was replaced by a corresponding moiety to construct compounds **1** and **3–11** (Table 1). A methyl group was introduced on the nitrogen atom at the 3-position of the imidazolidine ring of imidacloprid (**12**) to give compound **13**. For compounds **16–18**, the nitrogen atom at the 3-position of the imidazolidine ring of imidacloprid (**12**) was replaced by a methylene group, an oxygen atom and a sulfur atom, respectively. The 2-nitromethylene-1,3-perhydrodiazine moiety of compound **19** was derived from cyclohexene, because a partially planar-ring structure is required for the construction of this compound. Carbon atoms at the 1- and 3-positions of cyclohexene were replaced by nitrogen atoms and a nitromethylene moiety was introduced to the carbon atom at the 2-position. The 6-Cl-pyridylmethyl moiety was attached to the nitrogen atom at the 1-position of the perhydrodiazine to give compound **19**. Because the number of methylene groups between the two heterocyclic rings in compounds **4** and **5** is different from that of imidacloprid, the torsion angles between the two rings of compounds **4** and **5** were rotated from 0 to 360 degrees at 30-degree intervals using SYBYL in order to obtain the most stable conformers. The initial coordinates thus calculated were fully optimized by AM1 to give the most stable conformation for each compound. Initial conformations of *l*-nicotine and cytisine were obtained from the Cambridge Crystallographic Database[29,30] and that of *l*-anabasine was constructed from *l*-nicotine. Considering pK_a values of these amines, their protonated forms were modelled as active conformers (Fig. 4). As protonated *l*-nicotine, both *R*- and *S*-conformers at the 1-position were modelled and analysed. Conformations of these compounds were fully optimized by PM3 and the atomic charges were calculated using AM1. Based on the optimized stable conformers, the distance between the two nitrogen atoms of *l*-nicotine was 4.7 Å, which is in good agreement with the distance between the pyridyl nitrogen atom and the nitrogen atom at the 1-position of the imidazolidine ring of imidacloprid-**I–IV** (4·3 ~ 4·7 Å). One of the distances from the nitrogen atom at the 1-position of the imidazolidine ring to the respective two nitro-oxygen atoms, which was 4·6 Å, also was in good agreement, while the other was 4·2 Å.

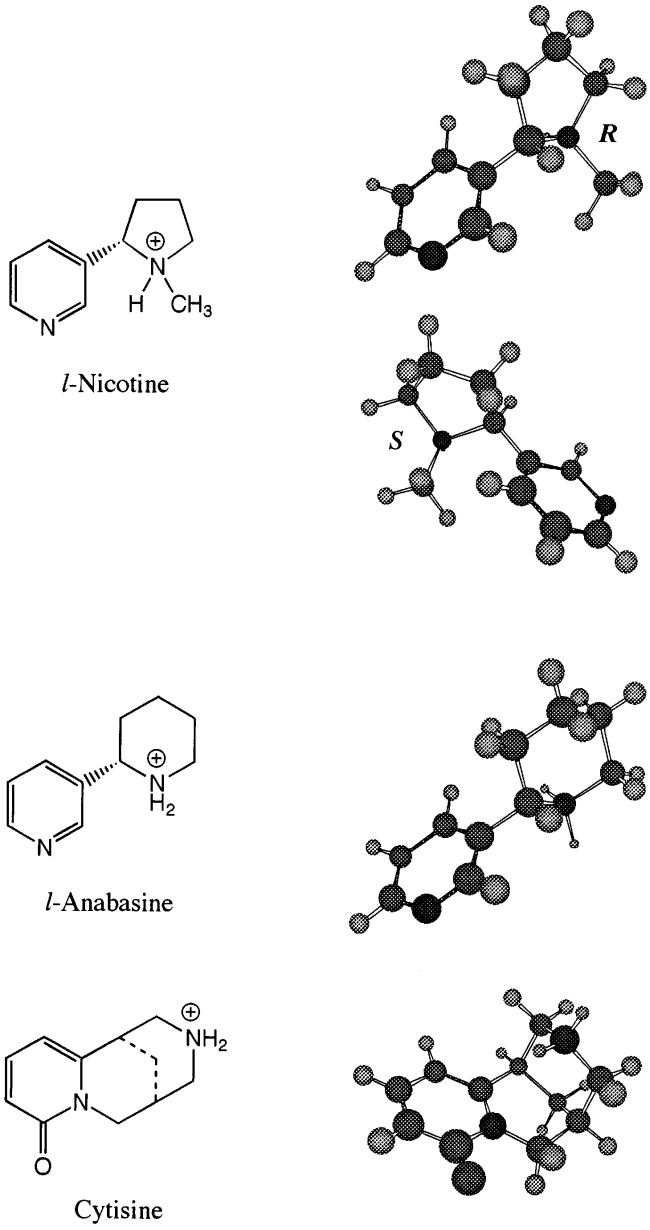


Fig. 4. Structures and optimized conformers of the protonated forms of *l*-nicotine, *l*-anabasine and cytisine.

2.3.2 Superposition

We assumed the fully optimized conformation of the molecules to be the active conformation for binding. The active conformation of imidacloprid was selected as the reference standard on which the other compounds were superposed for calculating the molecular-field descriptors. Compounds **1–19** were superposed in two ways (Fig. 5). Superposition-A was based on the binding model of Yamamoto *et al.*,[9] where the nitrogen atoms of the pyridine ring and at the 1-position of the imidazolidine ring, and the two carbon atoms adjacent to the nitrogen atom at the 1-position of the imidazolidine ring of imidacloprid were taken to be the key atoms for the superposition. Superposition-B was based on the model of Kagabu.[15] For this model, the nitrogen atom at the 1-position of the imidazolidine ring, one of the nitro-oxygen atoms, and the two atoms between the nitrogen atom and nitro group were taken to be the key atoms. In this case, the nitro-oxygen atom, which was 4·6 Å distant from the nitrogen atom at the 1-position of imidazolidine ring, was selected. For compounds **14** and **15**, which contain cyanoimino and cyanomethylene groups, respectively, the cyano-nitrogen atom was chosen as a key atom, rather than the nitro-oxygen

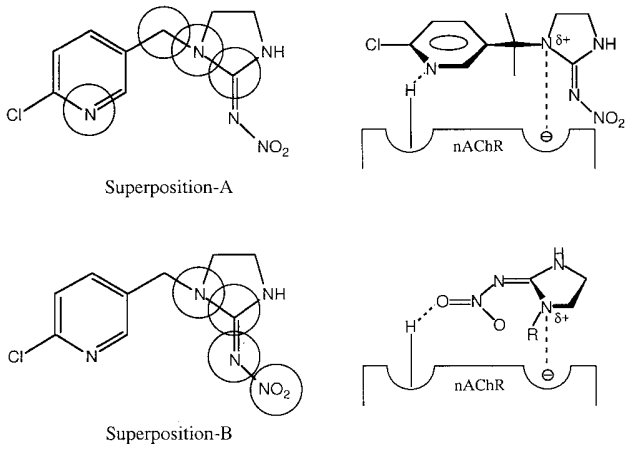


Fig. 5. Superpositions of imidacloprid and related compounds. Circled atoms of imidacloprid were superposed on the corresponding atoms of all other compounds with optimized conformations. Superpositions-A and -B are based on the binding models of Yamamoto *et al.*[9] and Kagabu,[15] respectively, shown at the right.

atom. The superpositions of *l*-nicotine, *l*-anabasine and cytisine on imidacloprid are illustrated in Fig. 6. The superposition was made so as to minimize the summation of the root mean squares of the distances of the atomic position of each compound from the corresponding atomic position of imidacloprid as far as possible.

2.3.3 *Correlation by CoMFA*

The analyses were done with the 'Advanced CoMFA' module of SYBYL. The superposed sets of active conformers were placed in a lattice of 18 Å × 24 Å × 21 Å ($X = -9$ to 9, $Y = -12$ to 12, $Z = -10$ to 11) with 1·5 Å spaces and the potential energy fields of each active conformer were calculated at the lattice intersections. For calculating the Coulombic electrostatic potential at each lattice point, the charge of +1·0 as a probe and the atomic charges for each of the molecules were used. The steric interaction (Lennard–Jones) potential at the lattice points was calculated using the sp^3-carbon atom as a probe. The data matrix was analysed by the partial least squares method.[31] In this procedure, an enormous number of 'original' independent lattice variables were transformed by linear combination so that all transformed variables were orthogonal, and the standard deviation between estimated and observed binding activity values were reduced as much as possible during the cross-validation tests. The transformed variables were usually so complex in composition that they were dealt with as latent variables. The results of the analysis are expressed as correlation equations with the number of latent variable terms used, and are displayed as contour diagrams of coefficients of the corresponding field descriptor terms at each lattice intersection, in order to show favourable and unfavourable potential regions. We initially selected the number of compounds in the set as the number of the cross-validation and then performed the analysis using the optimum number of latent variables, deduced from the cross-validation tests without actual cross-validation. The binding activities of compounds **1**–**19** in Table 1 were analysed for the four series of conformers **I**–**IV**, based on the two types of superposition as described above, for a total of eight series. A q^2 value of 0·3, the correlation coefficient obtained from the leave-one-out cross-validation, corresponds to the probability of chance correlation with an activity of less than 0·05.[32] Hence, correlation equations having q^2 values greater than 0·3 can be considered significant. Analyses for compounds, including three natural nAChR agonists (compounds **20**–**22**) were also conducted.

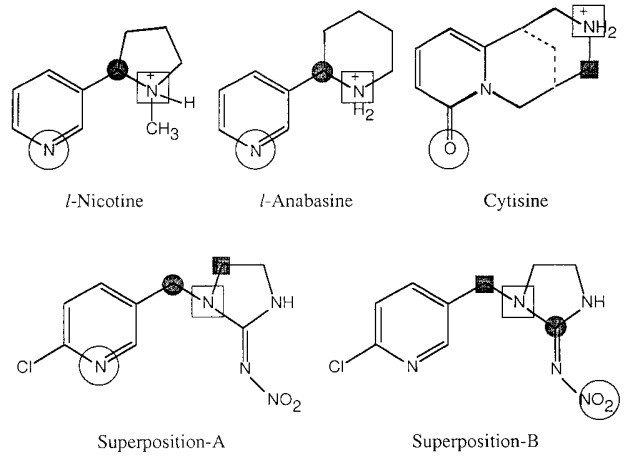


Fig. 6. Superpositions of *l*-nicotine, *l*-anabasine and cytisine on imidacloprid. The key atoms indicated by open circles, open squares and shadowed circles or squares were superposed on the corresponding atoms of imidacloprid.

3 RESULTS

3.1 Binding activity

The binding activities of the compounds are summarized in Table 1. The activity of compounds **4**–**7**, in which the distance between the nitrogen atom of the pyridine ring and the nitrogen atom at the 1-position of the imidazolidine ring is different from that in imidacloprid (**12**) was 15–700 times lower than that of imidacloprid itself. Substitution of the pyridine ring of compounds **1** and **2** with a benzene ring to give compounds **8** and **9**, respectively, decreased their binding activity by a factor of 70–160-fold, while substitution of a thiazolyl ring had no great effect on activity (**1** versus **10**). The binding activities of the compounds which contain a nitromethylene group were higher than those

for compounds having any other group at this corresponding position (**1** versus **11**; **2** versus **12**, **14** and **15**). When a methyl group was introduced on the nitrogen atom at the 3-position of the imidazolidine ring, a 150-fold decrease in binding activity was observed (**12** versus **13**). Conversion of the NH group at the 3-position of compound **2** to a CH_2 group (**16**) or a sulfur atom (**18**) had no significant effect on potency, whereas the compound in which the group was converted to an oxygen atom (**17**) showed a lower activity than compound **2**. Expansion of the imidazolidine ring by introducing a CH_2 group was slightly favourable to the activity (**2** versus **19**).

3.2 CoMFA results

The CoMFA statistical results for the binding activities are summarized in Table 2. In these equations, CN indicates the number of latent variables and s_{press} is the standard deviation obtained from the leave-one-out cross-validation. RC refers to the relative contribution of steric and electrostatic effects to variations in binding activity.

We initially analysed for 19 compounds (**1**–**19**) and obtained eqns (2)–(9). Among these, eqn (3) had the best quality in terms of q^2 value, although the number of components was smaller than the others, except for eqn (8). In these eqns, except for eqn (4) which is considered insignificant, electrostatic and steric fields are significant in rationalizing the activity variations.

These CoMFA results were further examined by adding compounds **20**–**22**. Because the *S*-conformer of the protonated *l*-nicotine had higher heat of formation than the *R*-conformer based on PM3 (*c.* 4 kcal mol^{-1}), the *R*-conformer only was included to derive eqns (10)–(17) (Table 2). In addition, the q^2 values and the CoMFA contour maps from the equations including the *S*-conformer were very close to those from the corresponding eqns (10)–(17) (data not shown). Among eqns (10)–(17), eqn (11), the counterpart of eqn (3), was the most significant. The $\log(1/K_i)$ values, calculated by eqn (11), are listed in Table 1.

Plate 1(a) and (b) represent the overlay of imidacloprid (**12**) with the major steric and electrostatic-potential contour maps, respectively, drawn according to eqn (11) after CoMFA. The green areas in Plate 1(a) indicate regions where submolecular bulk is well accommodated with an increase in binding activity, whereas the yellow areas indicate regions where the submolecular bulk is unfavourable for activity. The red areas in Plate 1(b) indicate regions where the more negative electrostatic interaction with the receptor binding site increases the activity, whereas the blue areas show regions where the reverse is the case. Figures similar to Plate 1(a) and (b) were also drawn according to eqn (3) (results not shown). These results indicate that

TABLE 2
Correlation Equations from CoMFA for the Binding Activity of Test Compounds $\log(1/K_i = A + [\text{CoMFA field terms}]$

							Cross-validated[b]		*RC*[c]		
Superposition	*Conformation*	*A*	*CN*[a]	*n*	*s*	r^2	s_{press}	q^2	*Steric.*	*Electro.*	*eqn no.*
A	**I**	1·65	4	19[d]	0·261	0·969	1·012	0·536	49·5	50·5	(2)
A	**II**	3·80	3	19[d]	0·451	0·901	0·857	0·643	37·9	62·1	(3)
A	**III**	[e]	4	19[d]	[e]	[e]	1·317	0·213	[e]	[e]	(4)
A	**IV**	2·64	4	19[d]	0·221	0·978	1·086	0·464	45·3	54·7	(5)
B	**I**	2·69	4	19[d]	0·443	0·909	1·108	0·430	48·7	51·3	(6)
B	**II**	4·24	4	19[d]	0·350	0·948	1·120	0·431	57·4	42·6	(7)
B	**III**	4·07	3	19[d]	0·594	0·828	1·071	0·442	52·9	47·1	(8)
B	**IV**	4·41	4	19[d]	0·423	0·919	1·130	0·420	53·8	46·2	(9)
A	**I**	2·25	5	22	0·260	0·966	1·064	0·429	41·2	58·8	(10)
A	**II**	3·90	4	22	0·396	0·916	0·884	0·581	33·0	67·0	(11)
A	**III**	[e]	5	22	[e]	[e]	1·256	0·204	[e]	[e]	(12)
A	**IV**	3·64	5	22	0·224	0·975	1·051	0·444	42·8	57·2	(13)
B	**I**	3·97	4	22	0·622	0·788	1·103	0·332	51·7	48·3	(14)
B	**II**	4·46	5	22	0·394	0·922	1·176	0·303	49·4	50·6	(15)
B	**III**	4·21	4	22	0·590	0·814	1·111	0·339	47·4	52·6	(16)
B	**IV**	4·99	5	22	0·404	0·918	1·282	0·172	48·7	51·3	(17)

[a] Number of components.
[b] Obtained from the leave-one-out cross-validation.
[c] Relative contribution (%).
[d] For compounds **1**–**19**.
[e] Not calculated.

eqn (11) which was derived by including three natural agonists with the estimated $\log(1/K_i)$ values is approximately the same level in significance to eqn (3).

Plate 1(a) and (b) show that both the steric and electrostatic regions mainly surround the nitroimino moiety and a part of the imidazolidine ring of imidacloprid as well. A positive electrostatic-potential region, favourable to activity, appears around the nitrogen atom of the nitro group and the carbon atom at the 2-position of the imidazolidine ring, whereas a negative eletrostatic region favourable to the activity is located at the oxygen atoms of the nitro group. A sterically forbidden region exists at one side of the plane, which includes the nitroimino moiety, and overlaps the positive electrostatic-potential region around the nitroimino moiety. A sterically permissible region appears at the other side of the plane.

Plate 1(a) also shows that there is a sterically forbidden region near the nitrogen atom of the pyridine ring of imidacloprid. Substitution of the pyridine ring of compounds **1** and **2** by a benzene ring to give compounds **8** and **9**, respectively, lowered the activity. This might be due to the hydrogen atom at the 3-position of the benzene ring protruding toward the unfavourable region.

The binding activity of compound **2**, which is the nitromethylene analog of imidacloprid (**12**), was about 30 times higher than that of imidacloprid (Table 1). A subtle difference in the bond angle of $=C\diagup\diagdown$ in compound **2** from that of $=N-$ in imidacloprid caused a difference in the conformations of the nitro groups. This could give rise to a situation where the nitro-oxygen atoms of compound **2** extend to the sterically favourable regions of ligands, thus resulting in a high binding activity. In this regard the nitro-oxygen atoms of compound **19** were closer to these regions than those of compound **2** (Plate 2(a)), resulting in the highest activity for compound **19**. Compound **14** which has a $=N-$ moiety in place of $=C\diagup\diagdown$ in compound **2** showed lower activity for similar reasons to those for imidacloprid (**12**) described above. Furthermore, in compounds **14** and **15**, the NO_2 group in the nitroimine of imidacloprid (**12**) and nitromethylene of compound **2**, respectively, is replaced by a CN group. The negative charge of the cyano-nitrogen atom ($-0{\cdot}07$) which could interact with the electropositive region of the receptor was much smaller than that of the nitro-oxygen atoms ($-0{\cdot}3$ and $-0{\cdot}4$), and, as a result, these compounds would be more likely to have lower activities than imidacloprid (**12**) and compound **2**. The low activity of compound **15** may also be related to its low stability.[14]

Compounds **7** and **13** exhibited very low activity. Compound **7** and imidacloprid (**12**) were superposed, so as to make the nitrogen atom at the 4-position of the pyridine ring of compound **7** and the nitrogen atom at the 3-position of the pyridine ring of imidacloprid as close as possible. This superposition resulted in the oxygen atoms of the nitro group of compound **7** invading the sterically unfavourable and electrostatically positive regions. The nitro group of compound **13** appears to be twisted, as the result of its interaction with the methyl group of the $\diagup\!\!>$N-Me moiety, and, hence, the nitro group comes close to the sterically prohibited region (Plate 2(b)). The oxygen atom of the 1,3-oxazolidine ring in compound **17** also contained a twisted nitro group, resulting in a slightly lower activity. The conformations of compounds **16** and **18** are nearly identical to that of compound **2**.

4 DISCUSSION

Nerve activity of imidacloprid and related compounds, log(1/EC), where EC is the concentration required to increase the frequency of spontaneous discharges in American cockroach nerve preparations, has been measured.[14] We previously reported eqn (18),[14] which demonstrated the relationship of the nerve activity to the binding activity, $\log(1/IC_{50})$, where IC_{50} was determined by Liu *et al.*[11] as the concentration of the compounds for 50% reduction in [^{125}I]α-BGTX binding to the house-fly head membranes.

$$\log(1/EC) = 0{\cdot}427(\pm 0{\cdot}145)\log(1/IC_{50}) + 0{\cdot}936(\pm 0{\cdot}308)\log P + 3{\cdot}446(\pm 0{\cdot}633)$$
$$n = 16,\ s = 0{\cdot}370,\ r = 0{\cdot}930,\ F(2, 13) = 41{\cdot}7 \quad (18)$$

In this equation, P is the partition coefficient in a 1-octanol/water system.[14] In a similar manner, we analysed the relationship between the nerve activity and the present binding activity, $\log(1/K_i)$, to give the following equation.

$$\log(1/EC) = 0{\cdot}505(\pm 0{\cdot}155)\log(1/K_i + 1{\cdot}190(\pm 0{\cdot}293)\log P + 2{\cdot}061(\pm 0{\cdot}995)$$
$$n = 15,\ s = 0{\cdot}310,\ r = 0{\cdot}948,\ F(2, 12) = 52{\cdot}9 \quad (19)$$

Compounds **15** and **17** were not included in eqn (19) because their log P values were not available. Compounds **4** and **7** were also excluded because their measured log(1/EC) values were much higher than those predicted from eqn (19). In the equation, in which compounds **4** and **7** were included, $s = 0{\cdot}606$ and $r = 0{\cdot}784$. A plausible explanation for this result may be that these two compounds raise the nerve effect via an alternative mechanism toward the nerve preparation. Equation (19) implies that the higher the binding affinity and the

greater the hydrophobicity, the higher is the nerve activity. The slope of the binding activity in eqns (18) and (19) shows that the binding of the compounds to the receptors is stoichiometrically responsible for about 50% of the neuroexcitatory effect. The slopes of the log *P* term in these two equations, which are close to unity, suggest that the term represents the perturbation of nerve membrane by the compounds, but not their interaction with the receptor.[33]

To clarify the physicochemical factors which affect the binding activity of imidacloprid and related compounds, CoMFA was used. The addition of the log *P* term to the CoMFA equations shown in Table 2 did not improve the correlations (data not shown). This suggests that the log *P* term in eqns (18) and (19) does not reflect hydrophobic interactions with receptors. The CoMFA results were most significant when the series of imidacloprid-**II** were superposed using the superposition method A (Fig. 5), suggesting that imidacloprid-**II**, one of the four conformers, may represent the active structure. The result is consistent with the binding mode proposed by Yamamoto *et al.*,[9] i.e. the nitrogen atom at the 1-position of the imidazolidine ring and the nitrogen atom of the pyridine ring interact with house-fly nAChR, but not an oxygen atom of the nitro group, as was proposed by Kagabu.[15] The nitroimino and imidazolidine moieties of the compounds appears to be surrounded by a sterically and electrostatically sensitive region of receptors (Plate 1). The nitrogen atom of the pyridine ring, however, is not surrounded by such sensitive fields (Plate 1), despite the fact that this supports the binding mode proposed by Yamamoto *et al.* These findings suggest that the nitro group is important for binding from the standpoint of electrostatic and positional effects. Variations in the binding activity of test compounds can be largely explained by the structural and conformational states of this moiety. The position of the nitrogen atom in a pyridine ring probably influences the positioning of the nitromethylene group, resulting in a change in the binding activity. However, it should be noted that these regions do not affect the importance of common structural features of the compounds, since the CoMFA method can only correlate variations in activity with structural differences between compounds, as pointed out by Agarwal *et al.*[32]

Despite the lack of nitro group, eqn (11) predicted the binding activity of three natural nAChR agonists **20**–**22** well. This strongly suggests that the binding site of imidacloprid and related compounds is similar to that of these natural agonists. The partially positive electrostatic site of the imidazolidine ring and the nitroimino moiety may have a role similar to cations of the nicotine-like agonists in binding with nAChR.

Nakayama and Sukekawa[34] extended the concept of molecular similarity indices and defined the similarity scores in a three-dimensional space (Sukekawa, M., 1996, pers. comm.). They analysed the correlation between structures of neonicotinoid insecticides and their receptor-binding activity which was measured by Tomizawa *et al.*[8] by using similarity scores for model compounds. They concluded that the nitroimino and imidazolidine moieties are surrounded by electrostatically favourable regions and the sterically favourable and forbidden regions. Our CoMFA results are similar to theirs.

Acetylcholine binds to the α-subunits of the nAChR in the electroplaque membranes of *Torpedo* rays to open the ion channel.[35] A pair of cysteines and some aromatic residues are included in the ligand binding site in the α-subunits.[36–39] The quaternary ammonium group of ACh may interact with the electron-rich π systems of the aromatic rings of Phe, Tyr and Trp which can be viewed as both polar and hydrophobic.[40,41] Similarly, imidacloprid and related compounds may interact with the aromatic moieties of the receptor at the nitrogen atom of the imidazolidine ring.

The amino acid sequences of insect nAChRs have been determined by cloning studies.[42,43] Some important residues at their putative ACh binding sites were conserved, but other residues were different from those of vertebrates,[44] in contrast with α-subunits of rays which show a high homology to those of human muscle. The difference in sequence may be related to the selective toxicity of imidacloprid against insects. In addition to the ligand structure–activity analysis, binding studies of ligands with the receptor site should be conducted at a molecular level to reveal the binding model of imidacloprid and related compounds.

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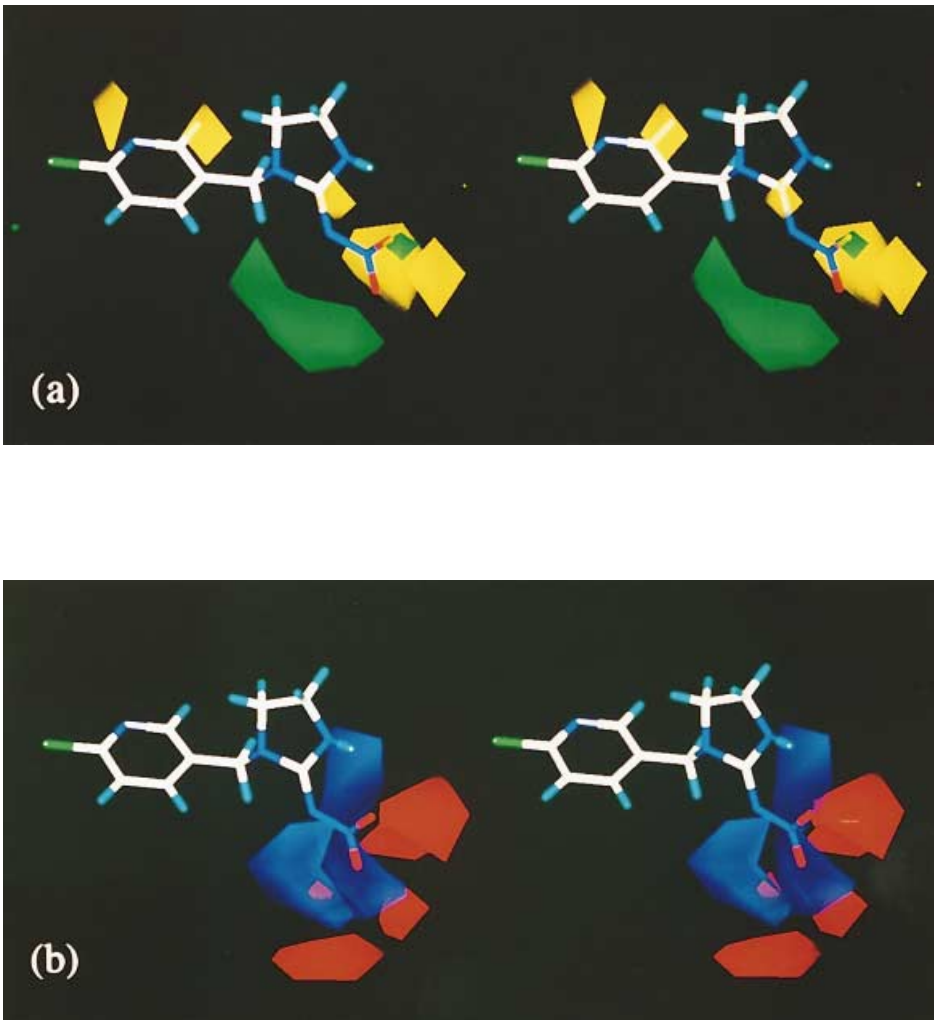

Plate 1. Stereoviews of contour diagrams of (a) steric and (b) electrostatic fields with imidacloprid (**12**) according to eqn (11). See text for an explanation of the colours.

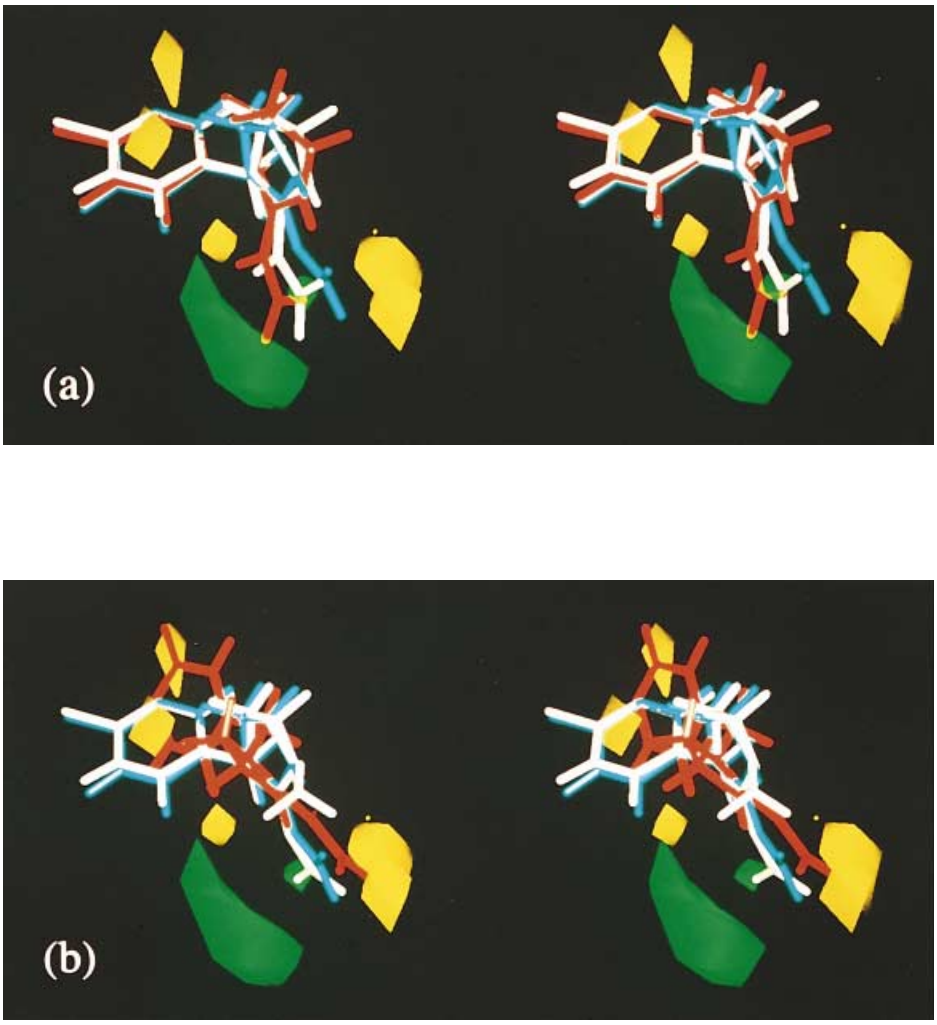

Plate 2. Stereoviews of contour diagrams of the steric fields according to eqn (11): (a) with imidacloprid (**12**) (light blue), compound **2** (white) and compound **19** (red); (b) with imidacloprid (**12**) (light blue), compound **7** (red) and compound **13** (white). The green areas show regions where the submolecular bulk is accommodated wherein the binding activity increases, while the yellow areas show the regions where the submolecular bulk decreases binding activity.